REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S): ARO 23768.1-LS-F
6a. NAME OF PERFORMING ORGANIZATION: University of Kentucky Department of Biochemistry	6b. OFFICE SYMBOL (If applicable): Biochemistry
7a. NAME OF MONITORING ORGANIZATION: U. S. Army Research Office	
6c. ADDRESS (City, State, and ZIP Code): Albert B. Chandler Medical Center Lexington, KY 40536-0084	7b. ADDRESS (City, State, and ZIP Code): P. O. Box 12211 Research Triangle Park, NC 27709-2211
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U. S. Army Research Office	8b. OFFICE SYMBOL (If applicable)
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAAL03-86-G-0032	
8c. ADDRESS (City, State, and ZIP Code): P. O. Box 12211 Research Triangle Park, NC 27709-2211	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
UNCLASSIFIED Efficient Low Cost Protein Factories: Expression of Human Proteins in Baculovirus Infected Insect Larvae

12. PERSONAL AUTHOR(S)
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13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Research	FROM 1986 TO 1989	1990, January 10	

16. SUPPLEMENTARY NOTATION
The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Molecular biology, genetic engineering. Production of recombinant proteins

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Human adenosine deaminase, a key purine salvage enzyme essential for immune competence, has been overproduced in Spodoptera frugipuda cells and in Trichoplusia ni (cabbage looper) larvae infected with recombinant baculovirus. The coding sequence of human adenosine deaminase was recombined into a baculovirus immediately downstream from the strong polyhedrin gene promoter. Approximately 60 hours after infection of insect cells with the recombinant virus, maximal levels of intracellular adenosine deaminase mRNA, protein and enzymatic activity were detected. The recombinant human adenosine deaminase represented 10% of the total cellular protein and exhibited a specific activity of 70 units/mg protein in crude homogenate. When the recombinant virus was injected into insect larvae, the maximum recombinant enzyme was produced four days post infection and represented about 2.0% of the total insect protein with a specific activity of 10-25 units/mg protein. (continued on reverse)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
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Block 19. Abstract (continued)

The recombinant human adenosine deaminase was purified to homogeneity from both insect cells and larvae and demonstrated to be identical to native adenosine deaminase purified from human cells with respect to molecular weight, interaction with polyclonal anti-adenosine deaminase antibody and enzymatic properties. A pilot purification yielded 8-9 mg of homogeneous enzyme from 22 larvae. The production of large quantities of recombinant human adenosine deaminase in insect larvae is inexpensive and rapid and eliminates the need for specialized facilities for tissue culture. This method should be applicable to large scale production of many recombinant proteins.

Efficient Low Cost Protein Factories: Expression of Human Adenosine Deaminase in Baculovirus Infected Insect Larvae

Final Report

Jeffrey A. Medin, Laura Hunt, Karen Gathy,
Robert K. Evans and Mary Sue Coleman

January 10, 1990

U. S. Army Research Office

Army Fellowship DAAL03-86-G-0032

University of Kentucky

FINAL REPORT

1. Foreward

This report covers the predoctoral research of Jeffrey A. Medin who has been supported from 1986-1989 on Army Fellowship DAAL03-86-G-0032. Mr. Medin has concentrated his studies on the enzyme adenosine deaminase, a purine salvage pathway enzyme. He has devised an efficient system for production of large quantities of recombinant enzyme and has initiated site-directed mutagenesis.

2. List of Appendixes

A. Appendix I

Manuscript: J.A. Medin et al. "Efficient Low Cost Protein Factories: Expression of Human Adenosine Deaminase in Baculovirus Infected Insect Larvae"

3. Report

A. The long-term goal of Jeffrey A. Medin is to produce altered human adenosine deaminases. He plans to mutagenize the enzyme at each of four tryptophan residues and study conformational changes in the altered proteins.

B. Results

An essential goal in this project was the creation of an efficient expression system for recombinant human ADA. Several bacterial expression systems had been used in our laboratory without great success. Jeff selected a eucaryotic expression system, baculovirus infected spo b tera frugipuda. In addition, he enhanced the usefulness of the system by overproducing a recombinant human protein in Trichoplusia ni larvae. The results of this work are described in detail in the accompanying manuscript. However, in summary this system appears to be an easy and inexpensive method for production of large quantities of recombinant proteins. With human ADA accumulation of several hundred mg of purified protein in a few weeks is entirely feasible. Mr. Medin is now extending this work to the production of altered human ADAs. He is also expressing other human proteins in the insect larvae.

C. Publications

J.A. Medin, L. Hunt, K. Gathy, R.K. Evans and M.S. Coleman "Efficient Low Cost Protein Factories: Expression of Human Adenosine Deaminase in Baculovirus Infected Insect Larvae" submitted to Proc. Natl. Acad. Sci. USA.

D. Jeffrey A. Medin (predoctoral student supported by the grant). Mr. Medin has been admitted to Ph.D. candidacy and should finish his degree in about a year.
Laura Hunt (undergraduate).
Mary Sue Coleman (predoctoral advisor)